

λ 4769, λ 4634, λ 4507, λ 4412, λ 4253.

Professor A. Herschel and Dr. von Konkoly pointed out long ago that the spectra of periodic meteors belonging to different swarms differ from each other, and the meteorites which come down to us differ greatly in their chemical constitution. It is not surprising to find the matter of the nucleus of this comet to exhibit a chemical difference from that of other comets.

In the diagram, the width of the continuous spectrum corresponds to the diameter of the nucleus. The bright bands extend into the coma on the side next the sun.

XVI. "On the Action of Heat upon the Contagium in the two forms of Septichæmia known respectively as 'Davaine's' and 'Pasteur's.'" By G. F. DOWDESWELL, M.A. (Cantab.), F.L.S., F.C.S., &c. Communicated by J. BURDON SANDERSON, M.D., LL.D., F.R.S., &c. Received June 15, 1882.

Professor Rosenberger, of Würzburg, has recently published the results of experiments,* by which he claims to have effectually sterilised by heat, the blood and exudation fluids of the rabbit in the two forms of septichæmia, known as those of Davaine and Pasteur; and he states that these fluids so sterilised, upon injection into other animals, were found to be infective, reproducing the disease with the recurrence of the specific organisms which characterise it: he therefore regards these organisms as having no causal connexion with the affections in which they are found, but as merely secondary or epiphenomenal. That this would be the necessary deduction from the experiments mentioned, if it were proved that the fluids had been effectively sterilised, is obvious; but the account published contains no details whatever of the methods employed, nor protocol of the experiments, so that it is impossible either to discuss them or to form a judgment as to the correctness of the conclusions. They, however, involve a question so important in respect to the theory of contagium vivum—the relations of these micro-organisms to disease—that it was determined to work out the subject on the basis indicated in Professor Rosenberger's paper, adopting such methods and precautions as appeared necessary.

* "Centralb. f. d. Med. Wiss.," 1882, No. 4, pp. 65-69.

PART I.—*Experiments upon Pasteur's Septichæmia in the Guinea-Pig.*

Guinea-pig No. 1.—0·7 cub. centim. of putrid ox-blood was injected with a Pravart's syringe, into the peritoneal cavity of a full-grown guinea-pig, which the next morning was found recently dead, rigor not having set in: round the place of injection there was some subcutaneous exudation, with destructive inflammation of the tissues of the abdominal wall, sections of which showed numerous Bacilli and Micrococci in the layers of connective tissue between the muscles. Acute peritonitis was found with a large exudation of serous fluid containing some extravasated blood-corpuscles, and deeply stained with their colouring matter. The fluid in this case was not very coagulable, differing in this respect from some others. The same day 0·5 cub. centim. of the peritoneal exudation fluid of No. 1 was injected into the subcutaneous tissue of the abdomen of guinea-pig No. 2, which, as the following day was Sunday, was not examined till Monday morning, when it was found dead, and in a much more advanced stage of decomposition than would have occurred normally in the same period. In all forms of septichæmia this rapid decomposition is invariably found. Guinea-pig No. 3 then received in similar manner 0·5 cub. centim. of the diluted subcutaneous exudation fluid of No. 2, which, likewise, was not coagulated. On the following morning No. 3 was found dead but still warm; the abdomen was infiltrated with a large quantity of subcutaneous exudation fluid, deeply stained with hæmoglobin, and containing some extravasated blood-corpuscles, as well as numerous active Bacilli and some Micrococci or spores. The serous fluid was not very coagulable; of this a portion was mixed with equal parts of normal saline solution, freshly made and boiled, containing in addition 1 per cent. of potassic carbonate, to render the serum alkaline and prevent coagulation upon boiling, which it did effectually. Vacuum tubes of about 10 millims. diameter and 10 centims. long, previously prepared, were then partially filled with this liquid by breaking their points under its surface; these were then placed one by one in a small flask of salt solution, to avoid frothing and splashing; this was then heated, and when the liquid was boiling freely, the tube enclosing it was resealed by the blow-pipe. The tubes were then placed in the hot air chamber, the temperature of which was gradually raised to 140° C., and maintained at nearly that point for one hour. No coagulation occurred upon heating, but it was found in subsequent experiments, that, inasmuch as the degree of dilution and alkalinity required to prevent this, varied in different cases, both with exudation serum and with blood, it was necessary in every instance to determine this point experimentally. The same evening guinea-pig No. 4 received by subcutaneous injection 0·3 cub. centim. of the exudation fluid of

No. 3, diluted and prepared as above mentioned, but unheated, while guinea-pig No. 5 received in similar manner 0·3 cub. centim. of the same fluid superheated and cooled. The following day, twenty-five hours after injection, No. 4 was prostrate, in a state of collapse, and the next morning was found dead with the same symptoms and micro-organisms as in previous cases, while No. 5 was quite unaffected, feeding heartily and with temperature unaltered. This animal remained healthy and unaffected for some days, and subsequently died from an accidental cause. On examination no symptoms whatever were found, either of septichæmia or peritonitis; there was no exudation of any sort and the blood was free from organisms. The following day the exudation fluid of No. 4 was diluted and rendered alkaline in the same manner as in the previous experiments, enclosed in tubes and superheated as before; of this 1·0 cub. centim. was injected into the subcutaneous tissue of guinea-pig No. 6, which, notwithstanding the large quantity it received, remained totally unaffected and was ultimately killed.

Some cultivation experiments were then made in different nutrient fluids, both with the sterilised and the unsterilised exudation serum, the result was that in all cases excepting one, in which there was reason to believe that the result was due to accidental contamination, no development of organisms occurred with the sterilised fluid, which it invariably did with the unsterilised.

In the septichæmia of Pasteur, the characteristic organism is a *Bacillus*, somewhat similar morphologically to the *B. anthracis*, and one of the forms of the hay *Bacillus*, *B. subtilis* of Cohn, the ubiquitous organism which develops so readily and constantly in most organic infusions, from atmospheric or other contamination, and until the specific morphological characters of these organisms are better discriminated than they are at present, that is probably until there is a further substantial advance in our optical appliances, it is scarcely safe to draw any conclusion from their occurrence in cultivations, unless these are numerous and repeated, with rigorous control experiments.

In this case, *i.e.*, Pasteur's septichæmia, or the malignant œdema of Koch, in the guinea-pig, I have invariably found both in this, as in other series of experiments, that in animals examined immediately upon death there are none of the specific organisms (the *Bacillus* described,—which is so large that it can scarcely be overlooked) to be found in the blood, or in any of the organs of the infected animal, and that the blood is not infective. After death, however, they speedily invade the organs of the animal, the kidneys and the spleen being apparently the first attacked; hence the necessity in all these cases of examining the subject immediately after death.

PART II.—*Experiments upon the blood of Rabbits in the form of Septichæmia known as that of Davaine.*

This form of septichæmia in the rabbit, which has attracted so much attention, and been the subject of so many experiments since those first published by Davaine and by MM. Coze and Feltz, in 1869, is originated by the subcutaneous injection of a small quantity—a few drops—of putrid blood, usually that of the ox. Infection with the specific disease in this case, as in the parallel one of septichæmia in the mouse, is somewhat uncertain, and the law on which it depends has not been clearly determined. Usually, however, blood which is only a few days old,—three or four—is, as originally stated by Davaine, the most readily infective, but by no means constantly so. In the following experiments all the rabbits employed were young, and nearly of the same age and size, not quite full grown. The septichæmic blood used for injection was always diluted, generally with an equal bulk of freshly prepared and boiled normal saline solution; the quantities of blood given as injected, unless otherwise stated, are always those of the blood itself which was used, and not of the solution.

No. 1.—0·2 cub. centim. of putrid ox-blood some days old was injected into the subcutaneous tissue of the back of rabbit No. 1. In forty-eight hours afterwards the rectal temperature was found to be 106° F., and the animal died on the third day. In the blood from the heart, examined immediately after death, a few of the organisms characteristic of the disease* were found, and preparations made and stained by the Weigert Koch method, gave the same result.

Two hours after death 0·1 cub. centim. of the blood of No. 1 was injected into rabbit No. 2. Twenty hours afterwards the rectal temperature was 104° F., and the animal expired in my presence twenty-three hours after injection. Section was made almost immediately, within about five minutes after death, but the blood was found already partially coagulated in the cavities of the heart and in the vessels. The blood which remained fluid was quickly mixed with two parts normal saline solution, with the addition of 3 per cent. pot. carb., and 0·3 cub. centim. of this solution (=0·1 cub. centim. blood) was injected into the subcutaneous tissue of rabbit No. 3, which died just within twenty hours after injection. A portion of the dilute alkaline blood was then placed in tubes, by breaking their points, sealed at a red heat, underneath the fluid; they were then immersed in a small flask of saline solution, sealed while boiling, and placed in the hot air oven, the temperature of which was gradually raised to 140° C., and slowly

* These are fully described in the forthcoming number (for June, 1882) of the "Journ. Royal Micros. Society."

cooled ; it was maintained at a temperature of over 100° C. for upwards of one hour. It being then late in the evening, rabbit No. 4 was on the next morning injected with a Pravart's syringe full of the dilute superheated blood. This animal survived and was but slightly, if at all affected, notwithstanding the very large quantity of fluid injected. The following day there was no appreciable disturbance whatever, but on the third day slight pyrexia occurred, the animal's skin felt hot to the touch, and the rectal temperature rose to $100\cdot0^{\circ}$ F., which, however, sometimes occurs normally in rabbits kept in confinement. Subsequently, it remained totally unaffected for several days, as long as observed, and was then destroyed.

Some days afterwards, the blood of another rabbit of a subsequent generation of the same infection, immediately upon death, was mixed with two parts normal saline solution, and 2 per cent. pot. carb., this was inclosed in tubes, boiled, sealed, and heated to 120° C., in the same manner as before.

Of the alkaline dilute blood (unheated) further diluted up to 100,000,000 times, 0·6 cub. centim. ($=\cdot000000006$ cub. centim. of blood) was injected into the back of rabbit E, which died of septichæmia within twenty hours.

Of the dilute superheated blood, 0·6 cub. centim. ($=0\cdot2$ cub. centim. blood) was injected into rabbit F, which the next day was apparently unaffected, but died with the usual symptoms of septichæmia the following day, *i.e.*, thirty-nine hours after injection. In the blood were found the characteristic Bacteria, and it proved to be infective when injected in a small quantity into another rabbit. Upon this, the same day, a further portion of the same superheated dilute alkaline blood was injected in the same quantity (0·6 cub. centim.) into rabbit G. This rabbit remained totally unaffected, and survived as long as observed, twenty days after, although it had received upwards of 30,000,000 times the quantity of blood which had proved lethal to rabbit E within twenty hours.

Cultivation experiments have been made both with the superheated blood and with that in the natural state, taken upon death ; but as it was found that the Bacterium which occurs in these cases refuses to germinate in any of the various cultivating media employed, excepting only in the serum of ox-blood, and in that very sparsely and uncertainly, they were inconclusive ; and the greater part of the cultivating glasses inoculated with blood of both sorts, after being kept several days in the incubator at temperatures between 30° and 40° C., unstable as the solutions are, remain to this day—a month subsequently—perfectly pellucid and unaltered, save from some loss by evaporation.

On considering these results, it appeared possible that, in the case where the superheated blood proved infective, from the method of

filling the vacuum tubes, portions of the dilute blood drying on their sides, might have escaped perfect sterilisation by heat; it was therefore determined to repeat the experiments with tubes filled by a method which should avoid this possible source of error. Tubes were made of thick German glass, about $\cdot 3$ inch internal diameter, closed at one end and slightly drawn out three or four inches from the bottom; then the blood of rabbit H, which died in my presence, of transmitted infection, was immediately collected and mixed with three times its bulk of normal saline solution with the addition of 3 per cent. potassic carbonate. In this instance it was found that a less degree of either alkalinity or dilution would not prevent coagulation on heating. Of this blood further diluted up to the ten-thousandth degree, 0·6 cub. centim. ($= 0\cdot 00006$ cub. centim. of the un-mixed blood) was injected into the back of rabbit I, which died of septichæmia in twenty-seven hours; the characteristic organisms were found in the blood, which on inoculation into another rabbit proved to be infective. A portion of the same alkaline dilute blood was then placed in the tubes above-mentioned, 2 to 3 cub. centims. in each, by means of a capillary pipette introduced to the bottom, contamination of the sides being avoided; the tubes were then drawn out by the blow-pipe to a capillary point, boiled in salt solution and sealed while boiling. One tube that was heated separately to 140° C. burst, as had been the case in several other experiments previously; the remainder were thereupon heated to fully 100° C. for six hours, when the temperature was raised to 130° C., were maintained at that for one hour, and then gradually cooled. Of this dilute superheated blood, rabbit K received by subcutaneous injection in the back 0·6 cub. centim. ($= 0\cdot 15$ cub. centim. blood), and rabbit L received 1·1 cub. centim. of the same ($= 0\cdot 275$ cub. centim. blood), that is nearly five thousand times as much as rabbit I had received of the unheated blood. Both these rabbits K and L were unaffected in any manner whatever: there was no appreciable variation of the rectal temperature; they continued to feed well, and did not lose flesh. They were killed ten days subsequently; and no material inflammation at or around the place of injection, and no thickening whatever of the tissues which occurs so markedly in all cases of infection could be observed; there was only a slight stain, as if by the colouring matter of the blood, in the subcutaneous tissue near the spot of injection.

From the result of these experiments I conclude that the active virus of infection, both in the case of Pasteur's septichæmia—the malignant œdema of Koch—in guinea-pigs, and in Davaine's septichæmia in rabbits, is destroyed by the prolonged action of a sufficiently high temperature; that blood or exudation fluid so treated is not infective, nor in any appreciable manner toxical, when injected in moderate quantities (up to 1 cub. centim.) into other healthy

animals, while the same fluids unheated, are invariably and fatally infective in infinitely smaller quantities.

In Davaine's septichæmia in the rabbit, I have found throughout these experiments that the period of incubation is remarkably constant, death, after the first generation of infection by putrid blood, almost invariably occurring from about twenty to twenty-five hours, and consequently that if it does not occur within about that period, it may be concluded that infection has failed; it may die subsequently, as rabbits in confinement constantly do, more especially under the conditions in which they are kept in laboratories; but unless within about the period specified they do not die infected with specific septichæmia, the characteristic organisms are not found in the blood, nor is that blood infective; hence it is not necessary to observe such animals for more than a few days after inoculation.

These experiments were conducted in the laboratory at University College, with the co-operation of Dr. Burdon Sanderson, and at his suggestion.

XVII. "On the Development of the Enamel of the Teeth of Vertebrates." By EMILY NUNN. Communicated by Professor HUXLEY, F.R.S. Received June 14, 1882.

[PLATES 2-4.]

The question of the origin of the enamel of the teeth in vertebrate animals has been the subject of much discussion. It has been held—1st, that the enamel results from the calcification of the enamel cells (Tomes, Waldeyer, Edwards); 2nd, that it is formed by excretion from those cells (Kölliker, Hertwig, Leydig); and, 3rd, that it is not formed by these cells at all, but has the same origin as the dentine, whatever that may be.*

Again, there are numerous opinions concerning the nature and origin of the *cuticula dentis*:—1, that the cuticula is the persistent basement membrane itself (Huxley, Hertwig, Leydig); 2, that it is the altered enamel membrane; 3, that it is the external layer of cells of the enamel organ (Waldeyer); 4, that it is the metamorphosed *stratum intermedium* of the enamel organ (Löwve); 5, that it is the ends (*Deckeln*) of the cells of the enamel membrane (Köllman); 6, that it is a dermic structure (Tomes); 7, that it is an excretion of the cells of the enamel membrane (Kölliker).

* Huxley, "Tegumentary Organs," "Encyclopædia of Anat. and Phys.," vol. v, Sup., gives reasons for considering the whole tooth ecdemonic, but adds, "All these points can only be decided by a much more extensive series of investigations."